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Disclosure: Authors have nothing to disclose.

Reference

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PP391
Keratin/chitosan as novel grafts for peripheral nerve regeneration

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Introduction: For many years Peripheral Nerve Injuries (PNI) has been raising major concerns in regenerative medicine. Currently, the gold standard treatment for PNI is the autologous nerve grafting but it presents several drawbacks. Chitosan, along with keratin, for their good biocompatibility and physicochemical properties have been widely used as biomaterials in tissue engineering scaffolding. Many engineered biomaterials, natural or synthetic, have also been studied, but its poor architecture and lack of appropriate biological cues have been limiting nerve tissue regeneration [1]. In this study, an innovative combination of chitosan and keratin is obtained in order to create a novel nerve conduit (medical device) aimed at finding applications in the treatment of PNI.

Materials and methods: Chitosan/keratin membranes and nanofibers were produced using solvent casting and electrospinning techniques, respectively. Scaffolds were physicochemical and biologically characterized. Medical grade chitosan was supplied by Altakitin (Portugal). Keratin was supplied by Nanyang Technological University (Singapore). Phosphate Buffer Saline, Dichloromethane, Trifluoroacetic Acid, Ammonia 7N in methanol, phalloidin and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich.

Results: Keratin/chitosan membranes and nanofibers topography showed a rough surface, with ridges and pores. FTIR revealed characteristic peaks of keratin and chitosan in both membranes and electrospun nanofibers, suggesting the presence of both biomaterials in the blend solution. Contact angle measurements showed that nanofibers possess a higher surface energy as compared to the membrane, indicating nanofibers' higher hydrophilic properties. Regarding mechanical properties, both membranes and nanofibers showed mainly elastic behavior and a low ability to dissipate energy, but the latest showed higher stiffness. Regarding biological assays, it was observed that cell viability increased with culturing time in both chitosan/keratin membranes and electrospun nanofibers. Nanofibers with keratin in the blend revealed higher adhesion ability as compared to chitosan alone, although not in a significant way.

Discussion and conclusions: The present results showed that keratin/chitosan scaffolds are better than chitosan alone, as they do not show significant difference in cell viability, although keratin/chitosan nanofibers appeared to elicit a better response than chitosan nanofibers. These data, together with the fact that materials have appropriate physicochemical and mechanical properties, indicate the suitability of the scaffolds for Peripheral Nerve Regeneration.

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Reference

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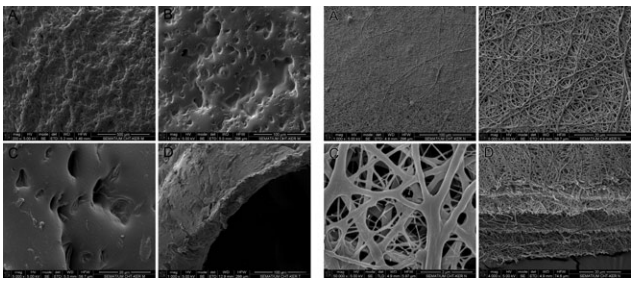

Figure 1. Chitosan/keratin membranes and electrospun nanofibers after neutralization process.

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Preparation and characterization of cross-linked collagen sponge with bone powder

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Introduction: Allograft bone is considered as the best bone substitution material used in bone defects. Since allograft bone powder is inconvenient to use, we chemically crosslinked collagen sponge with bone powder(CSB) and characterized the new type of tissue engineered scaffolds.

Materials and methods: The prepared CSB was cross linked by 50, 100 mM of EDC solution concentration and then lyophilized. The collagen sponges were characterized by SEM and IR spectrum in order to evaluate the porosity and water absorption ability. The cytotoxicity was assayed by MTT assay.

Results: The CSB surface was detected by SEM. Surface texture of collagen sponge was rough and many bone powder particles were observed (Fig. 1). L-929 cells were treated with CSB extracts according to ISO 10993-5(Control: cell only, Negative control (NC): DMSO, A: Without EDC treated, B: 50 mM EDC, 24-h CR, C: 50 mM EDC, 48-h CR, D: 100 mM EDC, 24-h CR, E: 100 mM EDC, 48-h CR). Because C group showed the lowest cell toxicity (Fig. 2), 50 mM EDC and 48-h cross-linking time were selected as the process condition and then BCS samples were prepared under this condition. All experiments were performed in triplicate. The results showed that the porosity of CSB was 94.8%, and the water absorption ability was 179.1%. The result of CSB FT-IR spectra confirmed that collagen characteristics were not influenced by EDC-treated (Fig. 3). CSB samples revealed typical bands of collagen mainly bands at 1650, 1560 and 1235/cm, characteristic of the amide I, II and III bands, respectively. Additionally, bands at 3450, 2850 and 1450/cm were observed, which represent the stretching of –OH, -CH_3 and pyrrolidine rings, respectively.

Discussion and conclusions: In order to obviate the inconveniences of using allograft bone powder, we developed a new form of bone substi-

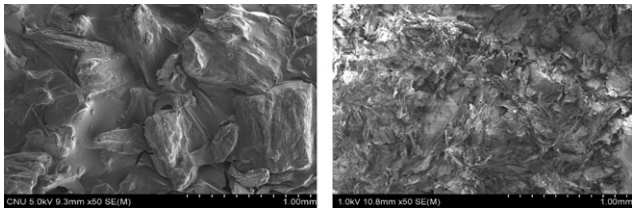

Figure 1. CSB surface image by SEM.